# INVERTASE IN HONEY

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## DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
THE FACULTY OF PURE SCIENCE,
COLUMBIA UNIVERSITY

BY

DAVID JOSAPHAT COHN, A.B., M.A.

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NEW YORK CITY

1924

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TO MY FATHER AND MOTHER

## ACKNOWLEDGMENT

The writer sincerely thanks Professor John M. Nelson for his sympathetic encouragement, and for his never-failing advice and assistance, both throughout the course of the work, and in the preparation of this dissertation.

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# Invertase in Honey

## Introduction

The invertase which has been the subject of the extensive researches of the past fifty years, has almost invariably been obtained from yeast. Sucrose splitting enzymes are, however, of widespread occurrence. Aside from yeast, several other species of fungus contain them [1, 2], and sucroclastic enzymes have also been found in the roots and fruits of higher plants [3-5], in certain forms of bacteria [6], and in the tissues and digestive secretions of some insects [7], birds [8], and mammals [9].

The question has naturally arisen from time to time, as to whether the invertases from such varied sources are identical, or whether each one is especially adapted to the peculiar conditions under which it is called upon to exert its influence.

In the case of other enzymes besides invertase, the same problem has, of course, also presented itself, and a number of comparative studies have been reported in the literature. The most far-reaching of these comparisons is that of Sherman and his students [10-16], who investigated the action of amylases derived from the pancreas, the saliva, malt, and takadiastase. The properties of these amylases do not exactly coincide. Nevertheless the investigators found enough parallelism in action, to lead them to the conclusion that, while not identical, all these amylases are similarly constituted, and that the mode of attack upon the substrate, starch, is the same in all cases.

Comparative studies have also been made, by Northrop [17] and others [18-20], upon the proteases obtained from the various digestive secretions of mammals, as well as from certain plants. These investigations have led to conclusions similar to those cited for the amylases.

The starches and the proteins are complex substances, and contain several kinds of hydrolyzable linkages within the molecule. One of the ways in which the enzymes from various sources, but specific to the same substrate, differ from one another, is that they do not affect corresponding linkages in the substrate in the same way. Thus, pepsin splits proteins only as far as the peptone stage; trypsin, on the other hand, hydrolyzes proteins all the way to amino-acids. In the case of the amylases from several plant and animal sources, the ratio of the amylolytic,

or starch splitting power, to the saccharogenic, or sugar forming power, varies between nine to one, and two to one.

These complexities leave the question open as to whether enzymes from different sources, specific to the same linkage within the same compound, bring about the hydrolysis of that linkage in exactly the same manner. For, since the hydrolysis of the several kinds of linkages within the starch or protein molecule all proceed simultaneously, it would be difficult to trace experimentally the progress of the reaction with respect to any one linkage singled out for study.

In order, therefore, to determine whether enzymes from different sources which are specific to the very same linkage in the same compound, act in exactly the same manner or not, it is desirable to compare the action of different preparations of enzymes acting upon a substrate in which there is only one linkage subject to the hydrolytic action. There are numerous cases of this kind, such as the hydrolysis of maltose by maltase, or of lactose by lactase. Several reports concerning studies of maltases and lactases [21] derived from different plant and animal sources are in the literature. In most instances, however, the enzyme preparations did not suffice for accurate and exhaustive investigations, and the errors involved in following the reaction were very large. Moreover, the effect of hydrogen ion concentration, which is of prime importance in enzyme work, was usually neglected. As a rule, too, the experiments were not planned with the object directly in view of comparing the actions of the enzymes from different sources, and hence hardly any strictly comparable data are available.

The hydrolysis of simple esters by the lipases, has been studied, however, with a view of comparing the action of enzymes from different sources. Thus, very recently, Willstætter and Memmem [22] have worked with pancreas and stomach lipases, and have come to the conclusion that certain apparent differences in their action are due to removable accompanying substances. The comparison was confined to a study of the effect of hydrogen ion concentration upon the rate of hydrolysis, and the experimenters were hindered by the difficulty that the stomach lipases which they used could only be obtained in preparations which were very weak, compared with those obtainable from the pancreas. There are, moreover, several difficulties inherent in the work with the esterases. The esters are only partially soluble in water, and this

puts limitations on the study of the effect of the enzymes upon solutions containing various substrate concentrations. Furthermore, the esters are partially hydrolyzed by the buffer solutions used to control the hydrogen ion concentration, and, besides, the enzymes need activating salts which are not the same in all cases. These factors interfere with the obtaining of exactly comparable conditions in the study of lipases from various sources.

Invertases, on the other hand, offer marked advantages for comparative studies of this kind. Their substrate, cane-sugar, also contains only one linkage which these enzymes attack. But cane-sugar, in contrast to the substrates mentioned above, is easily soluble in water, and readily obtainable in a high degree of purity. Moreover, the course of the inversion of sucrose can be very conveniently and accurately followed by means of the polariscope. A further distinct advantage that a study of invertases for comparative purposes presents, is that one invertase, that derived from bottom yeast, has been the subject of a large number of investigations. Very much of this work, especially in recent years, has been done under well standardized conditions, and a large mass of detailed information concerning yeast invertase is therefore available, with which the results of investigations upon an invertase from another source may be compared.

But despite these advantages offered by a comparative study of invertases, the object of most investigations upon other sources than yeast has been merely to ascertain the presence of an invertase in the organism studied. This is in large measure due to the difficulties which have heretofore been encountered in obtaining invertase preparations suitable for extended work, from any source except yeast. Thus, while Falk [4] did considerable work upon the invertase present in bananas, and Euler [9] upon that present in the intestines of mammals, the natures of the invertase preparations used unfortunately were not such as to permit of as exact measurements as have been possible with yeast invertase.

Solutions of banana invertase are not clear. This eliminates the use of the polariscopic method, which is the most convenient for following sucrose inversions. Moreover, purification by dialysis, a standard method in enzyme work, is not applicable, because banana invertase gels during the process, and at the same time undergoes considerable modification of activity.

With intestinal invertase, the main difficulty lies in its extrac-

tion from the parent cells. Euler, working with invertase from this source, was unable to obtain the free enzyme, excepting in dilute and unstable solutions. In fact, he carried out most of his hydrolyses by immersing measured strips of intestine in his sugar solutions. Such a procedure involves experimental difficulties, and depends on the asumption that the enzyme is equally distributed over the intestine surface. Euler met with another difficulty, in that the intestinal tissue exerts a powerful influence upon the hydrogen ion concentration of the solutions in which it is immersed, and he was unable to keep his mixtures at a constant hydrogen ion concentration during the time required to carry his experiments to completion.

As a result, although these investigations indicate differences in the action of the several invertases, they are not entirely conclusive, and do not form a satisfactory basis for future studies, with the purpose of determining the reasons for the divergencies noted in the behavior of the enzymes.

For the present research an invertase was sought, which could be obtained, just like yeast invertase, in clear, stable solutions. A search of the literature pointed to honey as the most likely source. Honey had been variously reported to contain a sucroclastic enzyme, and although nothing had been done to ascertain its properties and mode of action, one investigator had indicated that an active precipitate could be obtained when honey was treated with alcohol[7].

A method has now been developed for obtaining clear, aquaeous solutions containing honey invertase, which are comparable to yeast invertase preparations both as regards clearness and stability. The crude preparations obtained by alcohol precipitation, are amenable to purification by dialysis, and by other methods which are used in yeast invertase work. The honey invertase preparations procurable in this way are, moreover, comparable in strength to those ordinarily employed in the study of yeast invertase.

These favorable properties cause honey invertase to lend itself readily to the duplication of previous experiments conducted with the yeast product. We have here a very fortunate case of an invertase obtained from an entirely different source, which can be studied with exactly the same methods of procedure as are applied in the case of yeast invertase. In the present study, there have been compared some of the characteristics of these two

enzymes, the one derived from the fungus, yeast, and the other from an animal source, the digestive secretions of an insect, the bee.

## EXPERIMENTAL PART

### The Preparation of Honey Invertase

It was found that the physical properties of the honey invertase preparations, especially the clearness, depended both on the methods of precipitation and filtration, and on the kind of honey used as the starting material. Attempts to obtain a sucroclastic enzyme from canned "Orange Blossom Honey" were entirely unsuccessful. From combs of "White Clover Honey" active preparations were isolated, but these were too cloudy to permit of solutions containing them being read in the polariscope. Continued efforts to clarify these preparations with charcoal or kaolin were in vain. Where clear solutions did result from this treatment, they were either inactive, or showed only slight activity. It was finally found, however, that clear, active preparations could be obtained from a product called "Buckwheat Honey". This material, which is very dark in color, is available in the open market in small combs weighing about 400 grams each, designed for table use.

These combs were thoroughly mashed, and treated with about 150cc each of distilled water. That part of the beeswax which rose to the surface was skimmed off; the remainder was separated by suction filtration. To the filtrate were added seven to ten volumes of ninety percent. ethyl alcohol. The precipitated gums settled fairly rapidly; most complete settling was found to occur when the full quantity of alcohol was added at one time. The supernatant liquid was then siphoned off, and what was left was drained through fluted filters. For this operation suction filtration is unsuitable, for when it is used a cloudiness results, which cannot be easily removed by subsequent treatment. The gummy residue was in the main insoluble in water, but when stirred with the latter and filtered, yielded clear solutions containing active invertase. If fairly active preparations are desired, not more than ten cubic centimeters of water to each comb originally taken, should be used for this washing.

The first preparation procured in this way, was employed for preliminary experiments, without purification. All subsequent preparations were dialyzed in colloidion bags against running

water, for from forty-eight to seventy-two hours, in order to remove any contaminating salts or invert sugar. All solutions were treated with small amounts of toluene to prevent bacterial growth.

When the above procedure is carried out, an average yield of about ten cubic centimeters of invertase solution is obtained from each comb. This amount of enzyme solution, when present in one hundred cubic centimeters of a ten per cent. sucrose solution at 25°C., at the optimum hydrogen ion concentration, brings about the inversion of five grams of sucrose in about two and one-half hours.

## A COMPARISON OF CERTAIN PROPERTIES OF THE INVERTASES IN HONEY AND YEAST

Since varying results have always been obtained in attempts to determine the chemical constitution of yeast invertase by direct analysis, and no definite correlation has been shown between the composition and activity of different yeast invertase preparations, it was thought best to avoid this method for the time being in the study of honey invertase. Instead, the experiments were devoted entirely to determining the characteristics of inversions in the presence of honey invertase, and, for purposes of comparison, those characteristics of honey invertase were studied, of which the analogues in the case of yeast invertase are best known. Such characteristics are:

1. The course of hydrolysis of cane-sugar with respect to time, at various hydrogen ion concentrations.
2. The range of hydrogen ion concentration over which the enzyme shows the greatest activity.
3. The stability of the enzyme at various hydrogen ion concentrations.
4. The effect of various initial sucrose concentrations upon the course of hydrolysis.

**Experimental Method:** Before dealing exhaustively with the results of the experiments with honey invertase, undertaken to determine the characteristics of its inversions enumerated above, it is necessary to describe briefly the procedure followed, which is an application of the standard methods employed in following enzymatic sucrose inversions.

Definite quantities of honey invertase preparations were added

to sucrose solutions of known concentration, at a temperature of 25°C. The hydrogen ion concentrations of these solutions were controlled by means of sodium citrate buffers, and determined electrometrically. At measured time intervals during the course of the hydrolyses, samples were withdrawn from each of the reaction mixtures, and treated with sodium carbonate to interrupt the inversion. The samples were then polarized to ascertain what part of the hydrolysis had taken place.

A few preliminary experiments showed that the activity range of honey invertase centers about a hydrogen ion concentration of about $10^{-6}$, and the investigations were therefore conducted at hydrogen ion concentrations varying to both sides of this point.

Several invertase preparations were used in these studies, each obtained from honey purchased at different times over a period of three months. The experiments performed with any one preparation, were partially repeated with one or more of the others. By this means the possibility was precluded that the phenomena observed were peculiar to any one preparation, and not generally characteristic of honey invertase. In the data, the following designations are used for these preparations:

"HA" (not dialyzed), "HB" (dialyzed 48 hours),
"HC" (dialyzed 72 hours), "HD" (dialyzed 60 hours).

## COURSE OF HYDROLYSIS OF SUCROSE IN THE PRESENCE OF HONEY INVERTASE

The results recorded in Tables I-IV, and plotted in Figures I-V, represent hydrolyses of ten per cent. sucrose solutions in the presence of honey invertase. The ten per cent. concentration was chosen, because the most extensive work in this laboratory with yeast invertase, was done with sugar solutions of this concentration. The hydrolyses were run at hydrogen ion concentrations ranging from $10^{-3.95}$ to $10^{-7.60}$. Four different invertase preparations were used, and the rate of hydrolysis was varied considerably by the adjustment of the amount of invertase taken for the experiments of each series. The preparation, "A", "B", "C", or "D", which was used for each series of hydrolyses, is recorded in the data, and on the graphs.

**Description of the Course of Hydrolysis:** Despite all the variations in conditions noted in the preceding paragraph, all these hydrolyses manifest certain outstanding features, which they have

in common. Thus, immediately after the incipiency of the inversion, there is a marked increase in the reaction velocity, which lasts until a maximum is attained—generally between ten to fifteen per cent. hydrolysis—and then a gradual fall in velocity sets in. Each curve in Figures I-V, therefore, shows first a portion (marked "X" in Figure V) convex to the abscissa, then a point of inflection ("Y"), and, finally, a concave portion (marked "Z").

This course of hydrolysis, which inversions with honey invertase follow, is very different from that followed by inversions in the presence of yeast invertase. Yeast invertase inversions of ten per cent. sucrose solutions proceed at a practically constant rate at the beginning of the reaction, instead of showing the marked increase in rate found at the beginning of honey invertase hydrolyses. After the first portion of the hydrolysis is complete, the rate of inversion with yeast invertase falls off, in a manner similar to the behavior of honey invertase inversions, but, as will be shown below, the rate of the decline in the velocity of hydrolysis differs in the case of the two invertases.

## TABLE I

## COURSE OF HYDROLYSIS AND HYDROGEN ION CONCENTRATION

5cc of Honey Invertase Prep., "HC", in 225cc of Solution

Sucrose conc.: 10g per 100cc Temperature: 25°C.

Samples: 25cc $Na_2Co_3$ (.1M): 5cc Polar. tube: 400mm.

| pH | Time in minutes | Observed rotation | Change in rotation | Per cent. hydrolyzed | k x $10^6$ |
|---|---|---|---|---|---|
| 3.95 | 0 | 25.90 | | | |
| | 67½ | 25.83 | 0.07 | 0.21 | 13.3 |
| | 135 | 25.67 | 0.23 | 0.69 | 22.2 |
| | 385 | 25.16 | 0.74 | 2.21 | 25.2 |
| | 1335 | 22.44 | 3.46 | 10.30 | 36.0 |
| | 2914 | 18.95 | 6.95 | 20.70 | 34.5 |
| | 5670 | 14.95 | 10.95 | 32.60 | 30.2 |
| | 8586 | 12.40 | 13.50 | 40.22 | 27.2 |
| | Final | -7.66 | 33.56 | | |

TABLE I (Continued)

| pH | Time in minutes | Observed rotation | Change in rotation | Per cent. hydrolyzed | k x $10^6$ |
|---|---|---|---|---|---|
| 4.25 | 0 | 25.90 | | | |
| | 67½ | 25.67 | 0.23 | 0.69 | 66.2 |
| | 135 | 25.41 | 0.49 | 1.46 | 70.9 |
| | 270 | 24.74 | 1.16 | 3.46 | 75.3 |
| | 1485 | 18.25 | 7.65 | 22.79 | 87.9 |
| | 2840 | 13.20 | 12.70 | 37.83 | 86.1 |
| | 4370 | 9.26 | 16.64 | 49.60 | 66.1 |
| | 7600 | 3.70 | 22.20 | 64.65 | 59.4 |
| | Final | -7.67 | 33.57 | | |
| 4.69 | 0 | 25.89 | | | |
| | 67½ | 25.89 | 0.29 | 0.86 | 54.8 |
| | 135 | 25.15 | 0.74 | 2.21 | 71.9 |
| | 270 | 24.10 | 1.79 | 5.33 | 89.3 |
| | 1060 | 17.77 | 8.12 | 24.20 | 114 |
| | 2500 | 10.57 | 15.32 | 45.64 | 106 |
| | 4045 | 5.90 | 19.99 | 59.70 | 97.6 |
| | 5850 | 2.49 | 23.40 | 69.71 | 88.8 |
| | Final | -7.66 | 33.55 | | |
| 5.23 | 0 | 25.89 | | | |
| | 67½ | 25.46 | 0.43 | 1.28 | 83.0 |
| | 135 | 24.85 | 1.04 | 3.10 | 102 |
| | 270 | 23.36 | 2.53 | 7.54 | 109 |
| | 1060 | 15.63 | 10.26 | 30.56 | 150 |
| | 2500 | 8.22 | 17.67 | 52.65 | 130 |
| | 4045 | 3.75 | 22.14 | 65.97 | 120 |
| | 5850 | 0.65 | 25.24 | 75.16 | 104 |
| | Final | -7.68 | 33.57 | | |
| 5.67 | 0 | 25.91 | | | |
| | 67½ | 25.34 | 0.57 | 1.70 | 111 |
| | 135 | 24.57 | 1.34 | 3.99 | 131 |
| | 270 | 22.75 | 3.16 | 9.42 | 159 |
| | 540 | 19.26 | 6.65 | 19.82 | 178 |
| | 1365 | 12.41 | 13.50 | 40.22 | 164 |
| | 3495 | 4.32 | 21.59 | 64.33 | 128 |
| | 5635 | 0.49 | 25.42 | 75.71 | 109 |
| | Final | -7.67 | 33.58 | | |

TABLE I (Continued)

| pH | Time in minutes | Observed rotation | Change in rotation | Per cent. hydrolyzed | k x $10^6$ |
|---|---|---|---|---|---|
| 6.25 | 0 | 25.91 | | | |
| | 67½ | 25.30 | 0.61 | 1.82 | 119 |
| | 135 | 24.55 | 1.36 | 4.05 | 133 |
| | 270 | 22.56 | 3.35 | 9.98 | 169 |
| | 515 | 19.52 | 6.38 | 19.01 | 178 |
| | 1535 | 11.53 | 14.38 | 42.84 | 158 |
| | 2825 | 6.32 | 19.59 | 58.37 | 135 |
| | 4460 | 2.51 | 23.40 | 69.71 | 119 |
| | 7620 | -1.45 | 27.36 | 81.56 | 99.3 |
| | Final | -7.63 | 33.54 | | |
| 6.65 | 0 | 25.90 | | | |
| | 67½ | 25.36 | 0.54 | 1.61 | 104 |
| | 135 | 24.76 | 1.14 | 3.37 | 110 |
| | 385 | 21.71 | 4.19 | 12.48 | 150 |
| | 1265 | 14.25 | 11.65 | 34.72 | 144 |
| | 1895 | 10.95 | 14.95 | 44.56 | 131 |
| | 3365 | 6.10 | 19.80 | 59.02 | 114 |
| | 5640 | 1.83 | 24.07 | 71.71 | 80.2 |
| | Final | -7.66 | 33.56 | | |

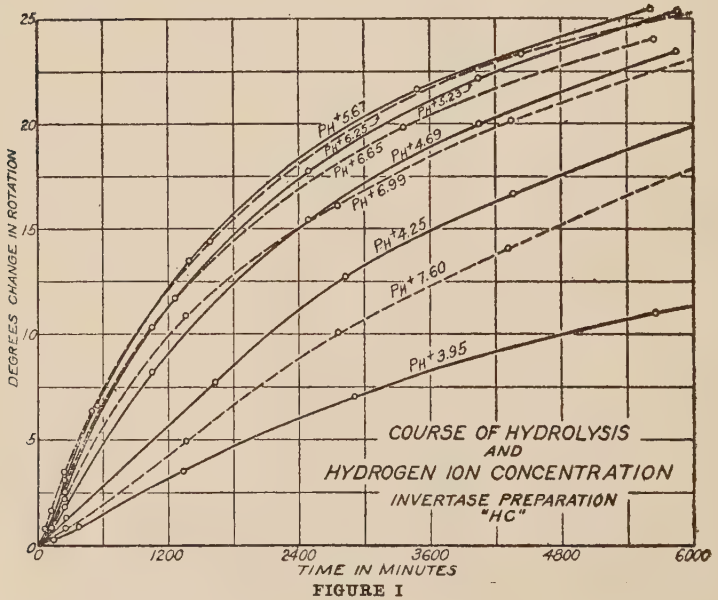



FIGURE I

TABLE I (Continued)

| pH | Time in minutes | Observed rotation | Change in rotation | Per cent. hydrolyzed | k x $10^6$ |
|---|---|---|---|---|---|
| 6.99 | 0 | 25.92 | | | |
| | 67½ | 25.46 | 0.46 | 1.37 | 88.9 |
| | 135 | 24.89 | 1.03 | 3.07 | 101 |
| | 270 | 23.58 | 2.34 | 6.97 | 116 |
| | 1385 | 15.20 | 10.72 | 31.94 | 121 |
| | 2765 | 9.89 | 16.03 | 47.75 | 102 |
| | 4340 | 5.74 | 20.18 | 60.12 | 92.0 |
| | 7492 | 0.96 | 24.96 | 74.30 | 78.8 |
| | Final | -7.66 | 33.58 | | |
| 7.60 | 0 | 25.91 | | | |
| | 67½ | 25.74 | 0.17 | 0.51 | 32.6 |
| | 135 | 25.57 | 0.34 | 1.01 | 32.6 |
| | 270 | 25.15 | 0.76 | 2.26 | 36.6 |
| | 1380 | 21.05 | 4.86 | 14.47 | 49.1 |
| | 2760 | 15.89 | 10.02 | 29.85 | 55.8 |
| | 4325 | 11.90 | 14.01 | 41.74 | 54.2 |
| | 7492 | 5.48 | 20.43 | 60.85 | 41.2 |
| | Final | -7.65 | 33.56 | | |

TABLE II

COURSE OF HYDROLYSIS AND HYDROGEN ION CONCENTRATION

8cc of Honey Invertase Prep., "HD", in 225cc of Solution
Sucrose Conc., 10g per 100cc Temperature: 25° C.
Samples: 25cc $Na_2CO_3$ (.1M) :5cc Polar. tube: 400 mm.

| pH | Time in minutes | Observed rotation | Change in rotation | Per cent. hydrolyzed | k x $10^6$ |
|---|---|---|---|---|---|
| 4.15 | 0 | 25.91 | | | |
| | 40 | 25.61 | 0.30 | 0.89 | 97.5 |
| | 80 | 25.20 | 0.71 | 2.12 | |
| | 120 | 24.74 | 1.17 | 3.49 | |
| | 180 | 24.06 | 1.85 | 5.51 | |
| | 300 | 22.43 | 3.48 | 10.38 | |
| | 525 | 19.44 | 6.47 | 19.28 | 177 |
| | 1435 | 11.07 | 14.84 | 44.23 | 176 |
| | Final | -7.67 | 33.58 | | |

TABLE II (Continued)

| pH | Time in minutes | Observed rotation | Change in rotation | Per cent. hydrolyzed | k x $10^6$ |
|---|---|---|---|---|---|
| 4.60 | 0 | 25.92 | | | |
| | 40 | 25.41 | 0.51 | 1.25 | 168 |
| | 80 | 24.76 | 1.16 | 3.46 | |
| | 120 | 23.98 | 1.94 | 5.78 | |
| | 180 | 22.75 | 3.17 | 9.45 | |
| | 300 | 20.17 | 5.75 | 17.14 | 272 |
| | 525 | 15.99 | 9.93 | 29.59 | |
| | 1435 | 6.46 | 19.46 | 57.98 | 262 |
| | Final | -7.67 | 33.59 | | |
| 5.25 | 0 | 25.91 | | | |
| | 40 | 24.97 | 0.94 | 2.81 | 308 |
| | 80 | 23.86 | 2.05 | 6.11 | |
| | 120 | 22.55 | 3.36 | 10.01 | |
| | 300 | 17.07 | 8.84 | 26.34 | 432 |
| | 525 | 12.31 | 13.60 | 40.52 | |
| | 1480 | 2.62 | 23.29 | 69.40 | 347 |
| | Final | -7.65 | 33.56 | | |
| 5.75 | 0 | 25.89 | | | |
| | 40 | 24.87 | 1.02 | 3.04 | 335 |
| | 80 | 23.44 | 2.45 | 7.30 | |
| | 120 | 21.89 | 4.00 | 11.92 | |
| | 180 | 19.62 | 6.27 | 18.68 | |
| | 300 | 15.88 | 10.01 | 29.83 | 513 |
| | 525 | 11.11 | 14.78 | 44.05 | |
| | 1435 | 2.05 | 23.84 | 71.03 | 375 |
| | Final | -7.65 | 33.54 | | |
| 6.15 | 0 | 25.91 | | | |
| | 40 | 24.86 | 1.05 | 3.13 | 345 |
| | 80 | 23.44 | 2.47 | 7.36 | |
| | 120 | 21.92 | 3.99 | 11.90 | |
| | 180 | 19.70 | 6.21 | 18.50 | |
| | 300 | 16.06 | 9.85 | 29.35 | 503 |
| | 525 | 11.34 | 14.57 | 43.42 | |
| | 1435 | 2.52 | 23.39 | 69.69 | 361 |
| | Final | -7.64 | 33.55 | | |

TABLE II (Continued)

| pH | Time in minutes | Observed rotation | Change in rotation | Per cent. hydrolyzed | k x $10^6$ |
|---|---|---|---|---|---|
| 6.42 | 0 | 25.90 | | | |
| | 40 | 24.82 | 1.08 | 3.22 | 355 |
| | 80 | 23.46 | 2.44 | 7.27 | |
| | 120 | 22.03 | 3.87 | 11.53 | |
| | 300 | 16.48 | 9.42 | 28.07 | 477 |
| | 525 | 11.95 | 13.95 | 41.57 | |
| | 1480 | 2.85 | 23.05 | 68.69 | 341 |
| | Final | -7.65 | 33.55 | | |
| 6.78 | 0 | 25.91 | | | |
| | 40 | 25.03 | 0.88 | 2.62 | 288 |
| | 80 | 23.92 | 1.99 | 5.93 | |
| | 120 | 22.70 | 3.21 | 9.57 | |
| | 180 | 20.92 | 4.99 | 14.87 | |
| | 300 | 17.79 | 8.12 | 24.20 | 401 |
| | 525 | 13.47 | 12.44 | 37.07 | |
| | 1435 | 4.56 | 21.35 | 63.62 | 306 |
| | Final | -7.66 | 33.57 | | |

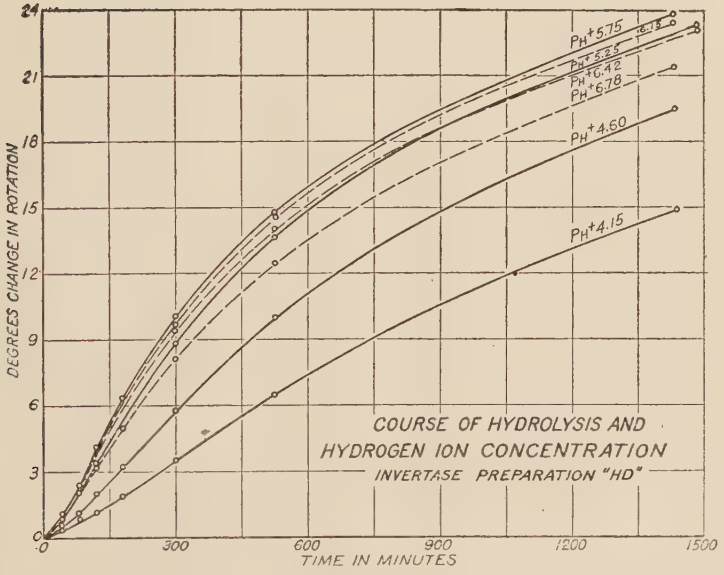



FIGURE II

## TABLE III

## COURSE OF HYDROLYSIS AND HYDROGEN ION CONCENTRATION

25cc of Honey Invertase Prep., "HB", in 250cc of Solution
Sucrose conc: 10g per 100cc Temperature: 25°C.
Samples: 25cc $Na_2CO_3$: 5cc (.1M) Pol. tube: 400mm

| pH | Time in minutes | Observed rotation | Change in rotation | Per cent. hydrolyzed | k x $10^5$ |
|---|---|---|---|---|---|
| 5.70 | 0 | 25.91 | | | |
| | 30 | 22.00 | 3.91 | 11.65 | 276 |
| | 60 | 17.25 | 8.66 | 25.81 | |
| | 90 | 13.69 | 12.22 | 36.42 | |
| | 140 | 9.60 | 16.31 | 48.61 | 353 |
| | 190 | 6.72 | 19.19 | 57.19 | |
| | 280 | 3.17 | 22.74 | 67.76 | |
| | 400 | 0.22 | 25.69 | 76.54 | 186 |
| | 600 | -2.64 | 28.55 | 85.09 | |
| | Final | -7.64 | 33.55 | | |
| 5.18 | 0 | 25.91 | | | |
| | 15 | 24.36 | 1.55 | 4.62 | |
| | 30 | 22.71 | 3.20 | 9.54 | 144 |
| | 60 | 19.85 | 6.06 | 18.06 | |
| | 90 | 15.39 | 10.52 | 31.34 | |
| | 140 | 11.06 | 14.85 | 44.37 | 174 |
| | 235 | 5.82 | 20.09 | 59.87 | |
| | 400 | 0.90 | 25.01 | 74.77 | 149 |
| | 600 | -2.32 | 28.23 | 84.12 | |
| | Final | -7.66 | 33.57 | | |
| 6.52 | 0 | 25.91 | | | |
| | 15 | 24.30 | 1.61 | 4.80 | |
| | 30 | 22.32 | 3.59 | 10.70 | 164 |
| | 60 | 19.52 | 6.39 | 19.04 | |
| | 90 | 15.50 | 10.41 | 31.02 | |
| | 140 | 11.65 | 14.26 | 42.49 | 179 |
| | 235 | 6.94 | 18.97 | 56.53 | |
| | 400 | 2.20 | 23.71 | 70.65 | 133 |
| | 600 | -1.00 | 26.91 | 80.21 | |
| | Final | -7.66 | 33.57 | | |

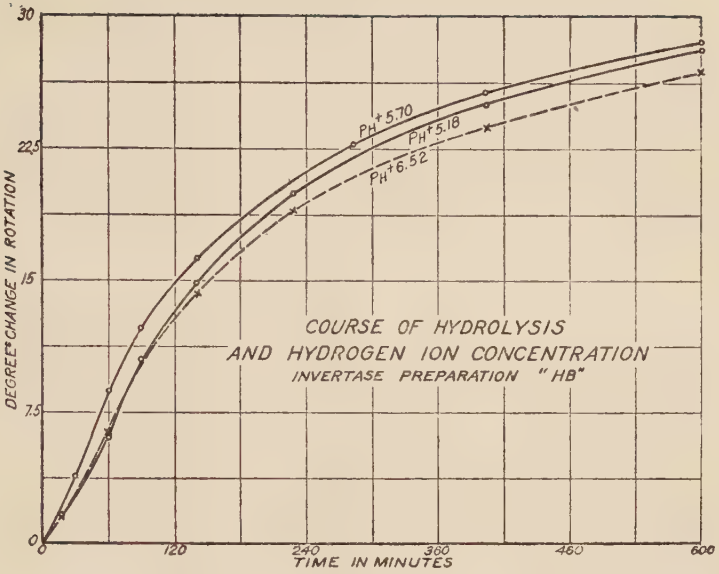



FIGURE III

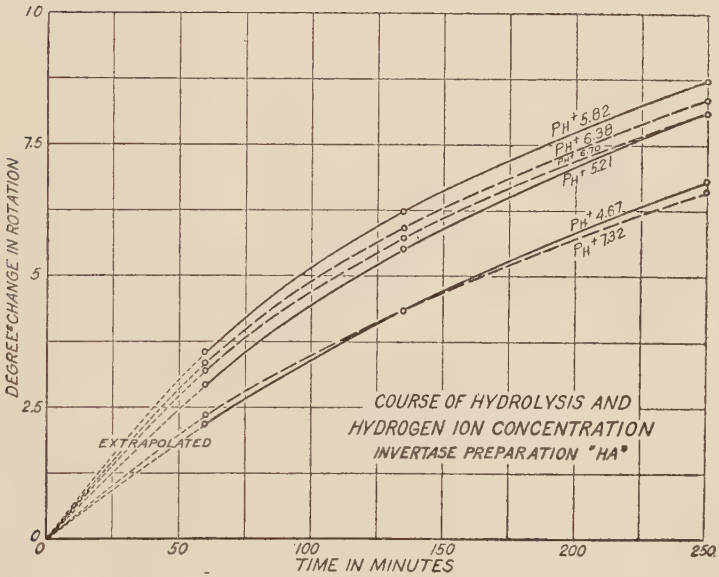



FIGURE IV

TABLE IV

COURSE OF HYDROLYSIS AND HYDROGEN ION CONCENTRATION

(10cc of Honey Invertase Prep., "HA") Temperature: 25°C.
(10g Sucrose ) Polar. tube: 200mm
(10cc of Buffer Solution ) Samples: 25cc
(80cc of $H_2O$ ) .1M $Na_2CO_3$: 5cc

Initial reading (extrapolated) 11.60°. Final: -3.40°

| pH | Time in minutes | Observed rotation | Change in rotation | Per cent. hydrolyzed | k x $10^5$ |
|---|---|---|---|---|---|
| 4.67 | 60 | 9.44 | 2.16 | 14.40 | 113 |
| | 135 | 7.25 | 4.35 | 29.00 | 110 |
| | 255 | 4.77 | 6.83 | 45.33 | 103 |
| 5.21 | 60 | 8.68 | 2.92 | 19.47 | 157 |
| | 135 | 6.09 | 5.51 | 36.74 | 147 |
| | 255 | 3.52 | 8.08 | 53.87 | 132 |
| 5.82 | 60 | 8.09 | 3.51 | 23.40 | 193 |
| | 135 | 5.37 | 6.23 | 41.53 | 173 |
| | 255 | 2.90 | 8.70 | 58.00 | 148 |
| 6.38 | 60 | 8.27 | 3.33 | 22.20 | 182 |
| | 135 | 5.68 | 5.92 | 39.47 | 162 |
| | 255 | 3.27 | 8.33 | 55.52 | 138 |
| 6.70 | 60 | 8.40 | 3.20 | 21.33 | 174 |
| | 135 | 5.90 | 5.70 | 38.01 | 154 |
| | 255 | 3.52 | 8.08 | 53.86 | 132 |
| 7.32 | 69 | 9.26 | 2.34 | 15.60 | 123 |
| | 135 | 7.28 | 4.32 | 28.80 | 109 |
| | 255 | 4.99 | 6.61 | 45.09 | 102 |

**The Monomolecular Equation Is Not Applicable:** Many workers in the invertase field apply the monomolecular equation in describing and comparing hydrolyses of sucrose with invertase. The monomolecular equation, though it fits acid hydrolyses of sucrose, is not so satisfactory for invertase hydrolyses, and its use in comparing such hydrolyses is necessarily crude. Nevertheless, since so much of the work recorded in the literature has been done on this basis, it was thought best to apply the monomolecular equation to honey invertase as well, so as to make the data for the latter available for comparison with the enzyme studies of the past.

In Column 6, of Tables I-IV, are tabulated the monomolecular "constants" obtained by an application of the equation to the

results given by honey invertase hydrolyses. The monomolecular constants rise rapidly at first, then diminished somewhat more gradually; hence, the inversions are not simple first order reactions.

The monomolecular constants calculated for yeast invertase hydrolyses have been shown almost invariably to rise steadily to the very end of the reaction, instead of showing the rise and fall characteristic of honey invertase inversions. This difference in behavior can be seen from the fourth column in the following examples:

Yeast Invertase[25]

10% Sucrose solutions pH 4.65 Polariscope tube: 200mm.

| Time in minutes | Observed Rotation | Per cent. Inversion | k x $10^5$ | n x $10^5$ |
|---|---|---|---|---|
| 0 | 12.03 | | | |
| 30 | 10.33 | 10.91 | 167 | 259 |
| 60 | 8.68 | 21.50 | 175 | 261 |
| 122 | 5.61 | 41.21 | 186 | 260 |
| 200 | 2.45 | 61.49 | 207 | 260 |
| 305 | -0.38 | 79.65 | 227 | 260 |

Honey Invertase

10% Sucrose solution. pH 5.70. Polariscope tube: 400mm.

| Time in minutes | Observed Rotation | Per cent. Inversion | k x $10^5$ | n x $10^5$ |
|---|---|---|---|---|
| 0 | 25.91 | | | |
| 30 | 22.00 | 11.66 | 179 | 276 |
| 60 | 17.25 | 25.81 | 216 | 317 |
| 140 | 9.60 | 48.61 | 295 | 353 |
| 190 | 6.72 | 57.19 | 194 | 247 |
| 400 | 0.22 | 76.54 | 157 | 186 |

**The Nelson-Hitchcock Equation Is Not Applicable:** In the case of the invertase from bottom yeast, Nelson and Hitchcock were able to evolve an empirical equation [23],

$$t=\frac{1}{n}\left(\log\frac{100}{100-p}+0.002642p-0.000008860p^2-0.0000001034p^3\right)$$

which accurately represents the course of inversion of ten per cent. sucrose solutions in the presence of this enzyme, over a wide range of hydrogen ion concentrations. It is quite evident from what was said above, that this equation does not hold for the very different course of reaction followed by inversions with

honey invertase. This is confirmed by the variation of the constants, "n", obtained by an application of the Nelson-Hitchcock equation to an hydrolysis with honey invertase, recorded in the fifth column of the table given in the preceding paragraph.

## COURSE OF HYDROLYSIS AND HYDROGEN ION CONCENTRATION

The manner in which inversions in the presence of honey invertase progress, is influenced considerably by the hydrogen ion concentration of the solution. This can be shown the best by means of the curves in Figure I, representing inversions conducted at hydrogen ion concentrations of $10^{-5.67}$ and $10^{-6.25}$, respectively. These curves show that the hydrolysis at a hydrogen ion concentration of $10^{-6.25}$, though starting off more rapidly than does that at $10^{-5.67}$, slows up much more than does the latter, once the initial stage has been passed; in fact, the curves representing these hydrolyses actually cross each other. An inspection of the rest of the curves in Figures I-IV, shows that the inversions in which the solutions are more alkaline than a hydrogen ion concentration of $10^{-6}$ decline more rapidly after the first stages of the hydrolysis are over, than do corresponding inversions at higher acidities. To bring this out more clearly in the graphic representation, all the curves in Figures 1-1V, which

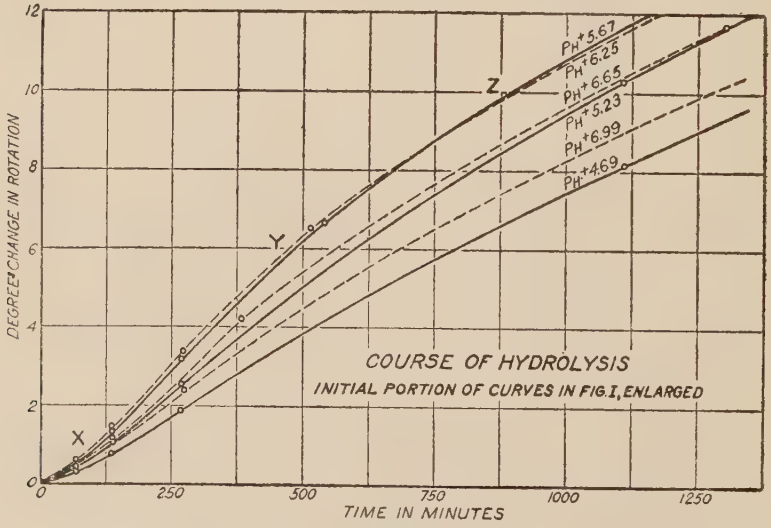



FIGURE V

represent inversions at hydrogen ion concentrations alkaline to $10^{-6}$ are drawn with broken lines, whereas the inversions at higher acidities are represented by plain line curves.

This non-uniformity at different hydrogen ion concentrations, of the course followed by inversions of ten per cent. sucrose solutions in the presence of honey invertase, is in marked contrast with the behavior of hydrolyses where yeast invertase is present. The Nelson-Hitchcock equation, cited above, has been found to hold for hydrolyses of ten per cent. sucrose solutions with the invertase derived from bottom yeast, over a wide range of hydrogen ion concentrations. All these yeast invertase hydrolyses, therefore, follow a uniform course. In other words, the curves representing them can be superimposed, as Nelson and Hitchcock showed, by simply adjusting the time scale. On the other hand, the curves representing honey invertase hydrolyses at various hydrogen ion concentrations are not superimposable.

## ACTIVITY AND HYDROGEN ION CONCENTRATION

The next point we will consider is the range on the hydrogen ion scale, over which the hydrolyses in the presence of honey invertase show the highest rate of inversion. In the case of yeast invertase, the relative magnitudes of the velocity constants given by the Nelson-Hitchcock equation present a convenient method for comparing the rates of inversion at different hydrogen ion concentrations. However, since honey invertase hydrolyses do not follow a uniform course at all hydrogen ion concentrations, it was impossible to derive an equation for honey invertase, similar to the Nelson-Hitchcock equation. It was, therefore, necessary to employ another method, used by Michaelis and Davidson[24], Nelson and Vosburgh[25], and others; namely, a comparison of the amount of hydrolysis which has taken place in a given time in each of the inversions.

The experimental data recorded in Tables I-IV, and graphically represented in Figures I-IV, can be used for this purpose. The times (in minutes) required for the hydrolysis of the first ten per cent. of the sugar present, were read off from Figures I and II. These numbers were then converted into values representing the relative velocities, by setting the smallest number equal to 100, and assigning reciprocally proportionate values to the others.

**The Optimum Hydrogen Ion Range Differs for Yeast and Honey Invertases:** The values resulting from the above procedure are tabulated in Tables V and VI, and plotted against hydrogen ion concentration in Figures VI and VII. They show that for honey invertase the range for optimum activity lies between hydrogen ion concentrations of $10^{-5.5}$ and $10^{-6.3}$.

This is quite different from the optimum range for yeast invertase, which lies between hydrogen ion concentrations of $10^{-4.4}$ and $10^{-5.0}$. The honey invertase is most effective in a region considerably more alkaline than the optimum range for yeast invertase.

## TABLE V

## ACTIVITY AND HYDROGEN ION CONCENTRATION

Honey Invertase Prep., "HC," data from Curves in Figure I.

| pH | Time for 10% Hydrol. (minutes) | Proportional numbers | Time for 35%-55% Hydrolysis | Proportional numbers |
|---|---|---|---|---|
| 4.25 | 695 | 41 | 2628 | 54 |
| 4.69 | 467 | 60 | 1821 | 76 |
| 5.23 | 348 | 81 | 1486 | 93 |
| 5.67 | 288 | 98 | 1388 | 100 |
| 6.25 | 282 | 100 | 1428 | 97 |
| 6.65 | 320 | 88 | 1628 | 85 |
| 6.99 | 384 | 73 | 2046 | 68 |
| 7.60 | 1002 | 28 | 3120 | 45 |

YEAST INVERTASE[26]

| pH | $n \times 10^5$ | Proportional numbers |
|---|---|---|
| 3.25 | 587 | 86.0 |
| 4.02 | 654 | 95.9 |
| 4.59 | 681 | 99.8 |
| 4.88 | 682 | 100.0 |
| 5.32 | 678 | 99.4 |
| 5.67 | 654 | 95.9 |
| 6.67 | 474 | 69.5 |

## TABLE VI

### ACTIVITY AND HYDROGEN ION CONCENTRATION

Honey Invertase Prep., "HD," Data from Curves in Figure II

| pH | Time for 10% Hydrol. (minutes) | Proportional numbers | Time for 35%-55% Hydrolysis | Proportional numbers |
|---|---|---|---|---|
| 4.15 | 310 | 35 | | |
| 4.60 | 204 | 53 | 664 | 62 |
| 5.25 | 128 | 84 | 460 | 90 |
| 5.75 | 110 | 100 | 412 | 100 |
| 6.15 | 110 | 100 | 424 | 97 |
| 6.42 | 108 | 98 | 468 | 88 |
| 6.75 | 152 | 71 | 572 | 72 |

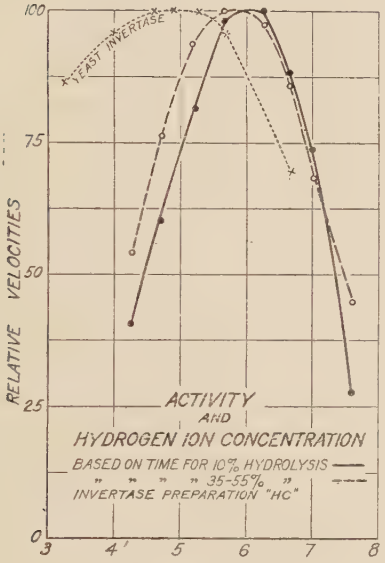



FIGURE VI

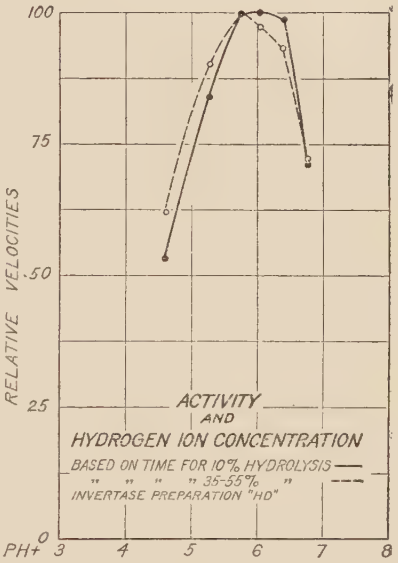



FIGURE VII

**The Optimum Hydrogen Ion Concentration for Honey Invertase Differs for Different Stages of the Hydrolysis:** It was shown above that hydrolyses of sucrose in the presence of honey invertase, do not follow the same course at all hydrogen ion concentrations. It was, in fact, pointed out that some of the curves in Figures I-IV, graphically representing the courses of inversions at various hydrogen ion concentrations, actually cross each other. Thus in the example used above, the hydrolysis at a hydrogen ion concentration of $10^{-6.25}$ (Figure I) at first proceeds

at a faster rate than the inversion conducted at $10^{-5.67}$, but after about twenty per cent. of the sugar present has been inverted the situation is reversed, the hydrolysis at $10^{-5.67}$ progressing faster than that at $10^{-6.25}$.

It then follows that if, instead of using the initial portions of the hydrolyses as the measure of the velocity, we use values based on the time consumed in the completion of some later portion, an entirely different result will be obtained for the optimum curve. This can be seen from the graphs in Figure VI. The plain line curve in this figure, represents the values obtained by plotting the relative initial velocities against hydrogen ion concentration. The curve drawn with a broken line is the result of plotting against hydrogen ion concentration, the relative velocities based on the time elapsed during the progress of the reaction from 35% completion to 55% completion, in the same inversions from which the relative initial velocities were calculated. The position of this curve, based on the later stage of the inversions, is appreciably more to the acid side with respect to the hydrogen ion scale, than is the curve based on initial velocities. Similar results were obtained with all the honey invertase preparations studied, but the extent of the difference in the optimum curves for different stages of the inversions does not appear to be exactly the same in all cases.

For yeast invertase hydrolyses, only one optimum curve is obtained, no matter what portion of the hydrolysis is taken as the criterion of the reaction velocity. This is to be expected from the fact, already mentioned, that the course of inversion in the presence of yeast invertase is unaffected by the hydrogen ion concentration of the substrate solution. It is interesting to note that the curve based on the rates of hydrolysis during the later portions of the inversions with honey invertase lies in an intermediate position on the hydrogen ion scale, between that occupied by the optimum curve for yeast invertase (dotted curve, Figure VI) and that for honey invertase, based on initial velocities.

It further appears from the curves in Figures VI and VII, that the magnitude of the effect upon the rate of hydrolysis with honey invertase, due to hydrogen ion changes, is not the same at all stages of the hydrolysis. Moreover, the same relative change in hydrogen ion concentration seems to cause larger differences in the rate of inversion with honey invertase, than with yeast invertase.

## STABILITY OF HONEY INVERTASE PREPARATIONS AND HYDROGEN ION CONCENTRATION

When it was first noticed that the decline in velocity of hydrolysis in the later stages of inversions with honey invertase is greater in solutions alkaline to a hydrogen ion concentration of $10^{-6}$, than in more acid solutions, it was thought that this might be due to instability of the enzyme. Accordingly inactivation tests were carried out (by a method described below) over a hydrogen ion range from $10^{-4.15}$ to $10^{-7.60}$. These tests showed that the enzyme is stable at hydrogen ion concentrations alkaline to $10^{-6}$, and hence instability is not the cause of the rapid falling off of the reaction velocity in this region.

The inactivation tests were carried out in accordance with the method employed by Nelson and Hollander.[27] To measured quantities of the invertase preparation, definite amounts of buffer were added to give the desired hydrogen ion concentration. With one part of the invertase buffer mixture a hydrolysis was immediately begun. The remainder of the mixture was allowed to incubate at 25°C. for twenty-five hours (in some cases the incubation period differed from this, as is noted in the data) and was used in an inversion begun the next morning.

The results, tabulated in Tables VII-IX, showed honey invertase to be stable in solutions alkaline to a hydrogen ion concentration of about $10^{-6}$, but to grow progressively more unstable as the hydrogen ion concentration is increased above this point. This instability of honey invertase in the more acid region, accounts for the rapidly diminishing reaction velocity of inversions conducted at hydrogen ion concentrations of $10^{-3.95}$, $10^{-4.15}$, and $10^{-4.25}$, which is noticeable in the curves representing these hydrolyses (Figures I and II).

In comparing the stability of honey and yeast invertases, it is found that yeast invertase can withstand greater acidities than can honey invertase. Nelson and Bloomfield [26] found no inactivation of the yeast invertase preparation they examined at 25°C, until a hydrogen ion concentration of $10^{-2.75}$ was reached; Nelson and Kerr [28] report some preparations which were partially unstable at a hydrogen ion concentration of $10^{-4.5}$. Both the honey and the yeast invertase appear to be stable in solutions alkaline to their respective optima ($10^{-6.0}$ and $10^{-4.9}$), and unstable in more acid solutions.

## TABLE VII

## STABILITY AND HYDROGEN ION CONCENTRATION

Honey Invertase Preparation, "HD."

10cc Invertase-Buffer mixture (containing 8cc Inv.) in 225cc solution

Sucrose conc.: 10g per 100cc — Temperature: 25° C.
Samples: 25cc — $Na_2CO_3$ (.1M) :5cc — Polar. tube: 400mm

| pH | Time in minutes | Obs'd rot. (I) (no incubation period) | Obs'd rot. (II) (25-hour incubation period) | Difference between I and II |
|---|---|---|---|---|
| 4.15 | 0 | 25.91 | 25.91 | |
| | 40 | 25.61 | 25.68 | 0.07 |
| | 80 | 25.20 | 25.40 | 0.20 |
| | 120 | 24.74 | 25.07 | 0.67 |
| | 300 | 22.43 | 23.32 | 0.89 |
| | 525 | 19.44 | 20.94 | 1.50 |
| 4.60 | 0 | 25.92 | 25.92 | |
| | 40 | 25.41 | 25.49 | 0.08 |
| | 80 | 24.76 | 24.87 | 0.11 |
| | 120 | 23.98 | 24.16 | 0.18 |
| | 300 | 20.17 | 20.62 | 0.45 |
| | 525 | 15.99 | 16.68 | 0.69 |
| 5.25 | 0 | 25.91 | 25.91 | |
| | 40 | 24.97 | 25.04 | 0.07 |
| | 80 | 23.86 | 23.94 | 0.08 |
| | 120 | 22.55 | 22.68 | 0.13 |
| | 300 | 17.07 | 17.21 | 0.14 |
| | 525 | 12.31 | 12.50 | 0.19 |
| | 1480 | 2.62 | 2.80 | 0.18 |
| 5.75 | 0 | 25.89 | 25.89 | |
| | 40 | 24.87 | 24.87 | 0.00 |
| | 80 | 23.44 | 23.50 | 0.06 |
| | 120 | 21.89 | 21.96 | 0.07 |
| | 300 | 15.88 | 15.97 | 0.09 |
| | 525 | 11.11 | 11.20 | 0.09 |
| | 1435 | 2.05 | 2.14 | 0.09 |

TABLE VII (Continued)

| pH | Time in minutes | Obs'd rot. (I) (no incubation period) | Obs'd rot. (II) (25-hour incubation period) | Difference between I and II |
|---|---|---|---|---|
| 6.42 | 0 | 25.90 | 25.90 | |
| | 40 | 24.82 | 24.84 | 0.02 |
| | 80 | 23.46 | 23.48 | 0.02 |
| | 120 | 22.03 | 22.05 | 0.02 |
| | 300 | 16.48 | 16.45 | -0.03 |
| | 525 | 11.95 | 11.94 | -0.01 |
| | 1480 | 2.85 | 2.85 | -0.00 |
| 6.78 | 0 | 25.91 | 25.91 | |
| | 40 | 25.03 | 25.00 | -0.03 |
| | 80 | 23.92 | 23.91 | -0.01 |
| | 120 | 22.70 | 22.68 | -0.02 |
| | 300 | 17.79 | 17.75 | -0.04 |
| | 525 | 13.47 | 13.45 | -0.02 |
| | 1435 | 4.56 | 4.53 | -0.03 |

TABLE VIII

STABILITY AND HYDROGEN ION CONCENTRATION

Honey Invertase Preparation, "HC".

10cc Invertase-Buffer Mixture (containing 5cc Inv.) in 225cc Solution

Sucrose conc.: 10g per 100cc Temperature: 25° C.
Samples: 25cc $Na_2CO_3$ (.1M) :5cc Polar. tube: 400mm

| pH | Time in minutes | Obs'd rot. (I) (no incubation period) | Obs'd rot. (II) (50-hour incubation period) | Difference between I and II |
|---|---|---|---|---|
| 4.25 | 0 | 25.90 | 25.89 | |
| | 67½ | 25.67 | 25.86 | 0.18 |
| | 135 | 25.41 | 25.73 | 0.31 |
| | 270 | 24.74 | 25.49 | 0.74 |
| 7.60 | 0 | 25.91 | 25.91 | |
| | 67½ | 25.74 | 25.76 | 0.02 |
| | 135 | 25.57 | 25.56 | -0.01 |
| | 270 | 25.15 | 25.13 | -0.02 |

TABLE IX

STABILITY AND HYDROGEN ION CONCENTRATION

Honey Invertase Preparation, "HA."

(20cc Invertase-Buffer Mixture, containing 10cc Inv.)
(10g Sucrose )
(80cc $H_2O$ )

Temp.: 25°C.

Samples: 25cc. $Na_2CO_3$(.1M) :5cc. Polar. tube: 200mm.

| pH | Time in minutes | Obs'd rot. (I) (no incubation period) | Obs'd rot. (II) (4-hour incubation period) | Difference between I and II |
|---|---|---|---|---|
| 6.38 | 0 | 11.60 | 11.60 | |
| | 100 | 6.49 | 6.49 | 0.00 |
| | 200 | 3.97 | 3.95 | -0.02 |
| | 400½ | 1.24 | 1.24 | 0.00 |

## COURSE OF HYDROLYSIS AND SUCROSE CONCENTRATION

In order to determine what changes in the course of reaction in honey invertase hydrolyses are induced by changes in the initial sucrose concentration, several series of inversions were conducted in which the initial sucrose concentration was varied from one to ten grams per hundred cubic centimeters, while the hydrogen ion concentration was kept constant (at $10^{-5.67}$ to $10^{-5.72}$, and $10^{-5.78}$ to $10^{-5.82}$) within the optimum range. The results of these experiments are recorded in Tables X-XII, and plotted in Figures VIII-X. From them it can be seen that the initial velocity of inversion increases with the increase of sucrose concentration until a point between 3% and 4% is reached. Thereafter, a further increase in sucrose concentration is not accompanied by an increase in initial velocity.

This characteristic of reaching an optimum sucrose concentration at about four per cent. is held in common by the honey and yeast invertases.[25] Yeast invertase hydrolyses also show increasing velocity with increase of sucrose concentration until a concentration of about four per cent. is reached. Above this concentration the reaction velocity in the presence of yeast invertase does not increase with added increments of sucrose, just as is the case with honey invertase.

Under the heading, "Course of Hydrolysis and Hydrogen Ion Concentration," it was noted that in hydrolyses of ten per cent. sucrose solutions with honey invertase, an increase in reaction velocity—absent in yeast invertase hydrolyses—sets in soon after the inversion is begun. The data and curves, given by the hydrolyses in which the sucrose concentration was varied (Tables X-XII, and Figures VIII-X), show that this increase in velocity took place in all those inversions in which the sucrose concentration was four per cent. or over. Below this sucrose concentration honey invertase hydrolyses run more nearly like those with yeast invertase, in that no increase in the velocity of hydrolysis sets in.

## TABLE X

## RATE OF HYDROLYSIS AND SUCROSE CONCENTRATION

5cc of Honey Invertase Prep., "HD", in 225cc of Solution

pH: 5.67—5.71 Temperature: 25°C.
Samples: 25cc $Na_2CO_3$ (.1M) :5cc Polar. tube: 400mm

| Sucrose conc. g per 100cc | Time in minutes | Observed rotation | Change in rotation | Difference in succes. readings |
|---|---|---|---|---|
| 1 | 0 | 2.55 | | |
| | 5 | 2.47 | 0.08 | 0.08 |
| | 10 | 2.40 | 0.15 | 0.07 |
| | 15 | 2.33 | 0.22 | 0.07 |
| | 30 | 2.10 | 0.45 | 0.23 |
| | 50 | 1.85 | 0.70 | 0.25 |
| | 90 | 1.37 | 1.18 | 0.48 |
| | 120 | 1.10 | 1.45 | 0.27 |
| 2 | 0 | 5.11 | | |
| | 5 | 5.00 | 0.11 | 0.11 |
| | 10 | 4.90 | 0.21 | 0.10 |
| | 15 | 4.80 | 0.31 | 0.10 |
| | 30 | 4.49 | 0.62 | 0.31 |
| | 50 | 4.08 | 1.03 | 0.41 |
| | 90 | 3.31 | 1.80 | 0.77 |
| | 120 | 2.79 | 2.32 | 0.52 |

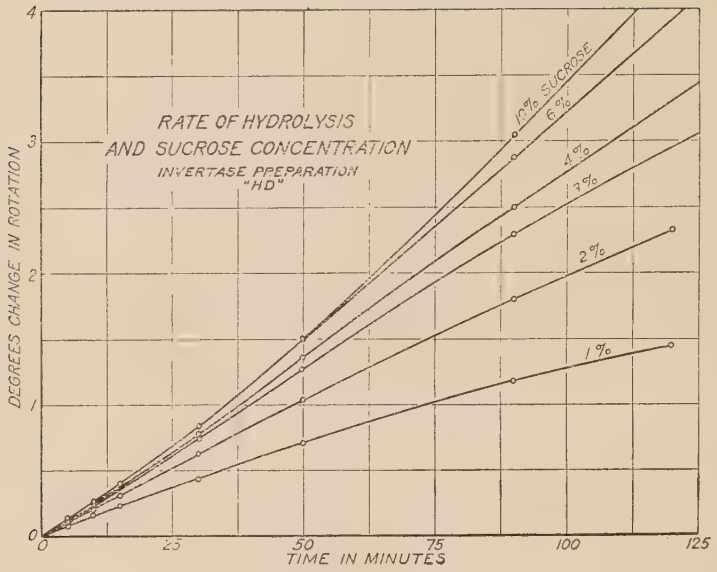


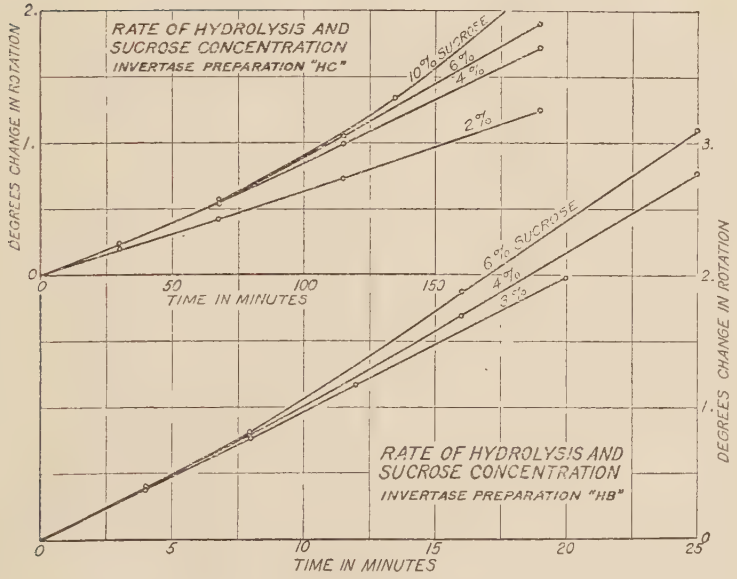

FIGURE VIII

## TABLE X (Continued)

| Sucrose conc. g per 100cc | Time in minutes | Observed rotation | Change in rotation | Difference in succes. readings |
|---|---|---|---|---|
| 3 | 0 | 7.73 | | |
| | 5 | 7.61 | 0.12 | 0.12 |
| | 10 | 7.48 | 0.25 | 0.13 |
| | 15 | 7.36 | 0.37 | 0.12 |
| | 30 | 6.98 | 0.75 | 0.38 |
| | 50 | 6.46 | 1.27 | 0.52 |
| | 90 | 5.44 | 2.29 | 1.02 |
| | 150 | 4.18 | 3.55 | 1.26 |
| 4 | 0 | 10.32 | | |
| | 5 | 10.19 | 0.13 | 0.13 |
| | 10 | 10.06 | 0.26 | 0.13 |
| | 15 | 9.93 | 0.39 | 0.13 |
| | 30 | 9.55 | 0.77 | 0.38 |
| | 50 | 8.95 | 1.37 | 0.60 |
| | 90 | 7.83 | 2.49 | 1.12 |
| | 150 | 6.23 | 4.09 | 1.60 |

TABLE X (Continued)

| Sucrose conc. g per 100cc | Time in minutes | Observed rotation | Change in rotation | Difference in succes. readings |
|---|---|---|---|---|
| 6 | 0 | 15.53 | | |
| | 5 | 15.40 | 0.13 | 0.13 |
| | 10 | 15.28 | 0.25 | 0.12 |
| | 15 | 15.15 | 0.38 | 0.13 |
| | 30 | 14.70 | 0.83 | 0.45 |
| | 50 | 14.02 | 1.51 | 0.68 |
| | 90 | 12.65 | 2.88 | 1.37 |
| | 150 | 10.58 | 4.95 | 2.07 |
| 10 | 0 | 25.92 | | |
| | 5 | 25.81 | 0.11 | 0.11 |
| | 10 | 25.69 | 0.23 | 0.12 |
| | 15 | 25.56 | 0.36 | 0.13 |
| | 30 | 25.09 | 0.83 | 0.47 |
| | 50 | 24.44 | 1.48 | 0.65 |
| | 90 | 22.90 | 3.02 | 1.54 |
| | 150 | 20.40 | 5.52 | 2.50 |




FIGURES IX and X

## TABLE XI

## RATE OF HYDROLYSIS AND SUCROSE CONCENTRATION

5cc of Honey Invertase Prep., "HC", in 225cc of Solution

pH: 5.67—5.72 Temperature: 25°C.

Samples: 25cc $Na_2CO_3$ (.1M) :5cc Polar. tube: 400mm

| Sucrose conc. g per 100cc | Time in minutes | Observed rotation | Change in rotation | Difference in succes. readings |
|---|---|---|---|---|
| 2 | 0 | 5.13 | | |
| | 30 | 4.93 | 0.20 | 0.20 |
| | 67½ | 4.71 | 0.42 | 0.22 |
| | 115 | 4.40 | 0.73 | 0.31 |
| | 190 | 3.90 | 1.23 | 0.50 |
| | 370 | 2.89 | 2.24 | 1.01 |
| | 1895 | 0.63 | 4.50 | 2.26 |
| 4 | 0 | 10.33 | | |
| | 30 | 10.11 | 0.22 | 0.22 |
| | 67½ | 9.78 | 0.55 | 0.33 |
| | 115 | 9.34 | 0.99 | 0.44 |
| | 190 | 8.62 | 1.71 | 0.72 |
| | 370 | 7.04 | 3.29 | 1.58 |
| | 1895 | 3.05 | 7.28 | 3.99 |
| 6 | 0 | 15.51 | | |
| | 30 | 15.28 | 0.23 | 0.23 |
| | 67½ | 14.95 | 0.56 | 0.33 |
| | 115 | 14.46 | 1.05 | 0.49 |
| | 190 | 13.61 | 1.90 | 0.85 |
| | 370 | 11.63 | 3.88 | 1.98 |
| | 1895 | 6.34 | 9.17 | 5.29 |
| 10 | 0 | 25.91 | | |
| | 67½ | 25.34 | 0.57 | 0.57 |
| | 135 | 24.57 | 1.34 | 0.77 |
| | 270 | 22.75 | 3.16 | 1.82 |
| | 540 | 19.26 | 6.65 | 3.49 |

## TABLE XII

## RATE OF HYDROLYSIS AND SUCROSE CONCENTRATION

10cc of Honey Invertase Prep., "HB", in 100cc of Solution

pH: 5.78—5.82 Temperature: 25°C.

Samples: 25cc $Na_2CO_3$ (.1M) :5cc Polar. tube: 400mm

| Sucrose conc. g per 100cc | Time in minutes | Observed rotation | Change in rotation | Difference in succes. readings |
|---|---|---|---|---|
| 3 | 0 | 7.72 | | |
| | 4 | 7.34 | 0.38 | 0.38 |
| | 8 | 6.94 | 0.78 | 0.40 |
| | 12 | 6.56 | 1.16 | 0.38 |
| | 20 | 5.75 | 1.97 | 0.81 |
| 4 | 0 | 10.33 | | |
| | 4 | 9.97 | 0.36 | 0.36 |
| | 8 | 9.56 | 0.77 | 0.41 |
| | 16 | 8.70 | 1.63 | 0.86 |
| | 25 | 7.60 | 2.73 | 1.10 |
| 6 | 0 | 15.53 | | |
| | 4 | 15.16 | 0.37 | 0.37 |
| | 8 | 14.74 | 0.79 | 0.42 |
| | 16 | 13.67 | 1.86 | 1.07 |
| | 25 | 12.44 | 3.09 | 1.23 |

## SUMMARY

A method has been developed for obtaining an invertase from honey, in clear, transparent solutions.

The course followed by the hydrolysis of sucrose in the presence of the invertase from honey has been studied under several different conditions, such as varying hydrogen ion and sucrose concentrations.

The invertases from the two sources show several distinct differences in the way they cause the hydrolysis of sucrose to proceed.

The course of hydrolysis with honey invertase, in the case of sucrose concentrations of four per cent. or higher, is marked by an increase in the inversion rate, soon after the reaction begins. No such initial increase in velocity is found in the case of the hydrolysis with yeast invertase.

After the first portion of the hydrolysis is completed, the rate of inversion with honey invertase drops off relatively more rapidly, than in the case of hydrolysis with yeast invertase.

The invertase from honey has an optimum activity range at hydrogen ion concentrations from $10^{-5.5}$ to $10^{-6.3}$, whereas the optimum range for the invertase from yeast lies between $10^{-4.4}$ and $10^{-5.0}$.

The optimum hydrogen ion concentration for the activity of honey invertase varies for different stages in the hydrolysis of sucrose, while in the case of yeast invertase it remains the same for all stages.

Both honey and yeast invertase are more stable in solutions somewhat alkaline to their respective optima, than in more acid solutions. Yeast invertase can withstand greater acidities than honey invertase, some preparations of the former being stable at hydrogen ion concentrations of $10^{-3.0}$, whereas the honey invertase preparations studied were partially inactivated at a hydrogen ion concentration of $10^{-5.8}$.

With increasing sucrose concentrations, both honey and yeast invertases show increasing initial velocities of hydrolysis, until an optimum sucrose concentration is reached. This optimum sucrose concentration is at about four grams per hundred cubic centimeters, in both instances.

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## VITA

David Josaphat Cohn was born in New York City on October 3, 1896. He attended the public schools of the city, and was graduated from the College of the City of New York in 1917, with the degree of Bachelor of Arts. In the same year, he entered upon graduate studies in the Department of Chemistry at Columbia University. During 1918, he was with the Chemical Warfare Service, and in 1919 returned to the University, where he received the degree of Master of Arts. He continued his graduate studies until late in 1921, serving for several terms as laboratory assistant. Thereafter he was for some time engaged in outside pursuits, and returned in 1923 to complete his studies for the degree of Doctor of Philosophy. He is a member of Sigma Xi.